mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 injected mice per group shall be challenged intraperitoneally 10–12 days after the second vaccination with a 0.2 ml dose containing 100–10,000 mouse $LD_{50}$, as determined by titration, of a suitable culture of *Pasteurella multocida*. All survivors in each group of mice shall be recorded 10 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.50, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test: *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of this section, the serial is unsatisfactory.

[40 FR 17004, Apr. 16, 1975, as amended at 42 FR 59487, Nov. 18, 1977; 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66785, Dec. 26, 1991]

### § 113.122 Salmonella Choleraesuis Bacterin.

Salmonella Choleraesuis Bacterin shall be prepared from a culture of *Salmonella choleraesuis* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella choleraesuis* fraction shall meet the applicable requirements in 9 CFR 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in 9 CFR 113.33(b).

The subcutaneous route shall be used when the product is in combination with Pasteurella Multocida Bacterin.

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Veterinary Services.

(2) At least three fivefold dilutions shall be made with the Standard and the same fivefold dilution shall be made for each Unknown. The dilutions shall be made in Phosphate-Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7 to 10 days after the second vaccination with a 0.25 ml dose containing 10–1,000 mouse $LD_{50}$ as determined by titration of a suitable culture of *Salmonella choleraesuis*. All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$RP = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.50, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP values obtained in the retests shall be determined. If the average RP is less than the required minimum, the serial is unsatisfactory. If the average RP obtained in the retests is equal to or greater than the required minimum, the following shall apply:

(i) If the RP obtained in the original test is one-third or less than the average RP obtained in the retests, the initial RP may be considered a result of test system error and the serial is satisfactory.

(ii) If the RP value obtained in the original test is more than one-third the average RP obtained in the retests, a new average shall be determined using the RP values obtained in all tests. If the new average is less than the minimum required in paragraph (c)(7) of

this section, the serial is unsatisfactory.

[43 FR 25077, June 9, 1978, as amended at 48 FR 31008, July 6, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66785, Dec. 26, 1991]

### § 113.123 Salmonella Dublin Bacterin.

Salmonella Dublin Bacterin shall be prepared from a culture of *Salmonella dublin* which has been inactivated and is nontoxic. Each serial of biological product containing *Salmonella dublin* fraction shall meet the applicable requirements in 9 CFR 113.100 and shall be tested for purity, safety, and potency as prescribed in this section. A serial found unsatisfactory by any prescribed test shall not be released.

(a) *Purity test.* Final container samples of completed product shall be tested for viable bacteria and fungi as provided in 9 CFR 113.26.

(b) *Safety test.* Bulk or final container samples of completed product from each serial shall be tested for safety as provided in 9 CFR 113.33(b).

(c) *Potency test.* Bulk or final container samples of completed product from each serial shall be tested for potency using the mouse test provided in this paragraph. A mouse dose shall be 1/20 of the least dose recommended on the label for other animals which shall not be less than 2 ml.

(1) The ability of the bacterin being tested (Unknown) to protect mice shall be compared with a Standard Reference Bacterin (Standard) which is either supplied by or acceptable to Veterinary Services.

(2) At least three tenfold dilutions shall be made with the Standard and the same tenfold dilutions shall be made for each Unknown. The dilutions shall be made in Phosphate-Buffered Saline.

(3) For each dilution of the Standard and each dilution of an Unknown, a group of at least 20 mice, each weighing 16 to 22 grams, shall be used. Each mouse in a group shall be injected intraperitoneally with one mouse dose of the appropriate dilution. Each mouse shall be revaccinated on day 14, using the same schedule.

(4) Each of 20 vaccinated mice per group shall be challenged intraperitoneally 7 to 10 days after the second vaccination with a 0.25 ml dose containing 1,000–100,000 mouse $LD_{50}$ as determined by titration of a suitable culture of *Salmonella dublin*. All survivors in each group of mice shall be recorded 14 days postchallenge.

(5) Test for valid assay: At least two dilutions of the Standard shall protect more than 0 percent and two dilutions shall protect less than 100 percent of the mice injected. The lowest dilution of the Standard shall protect more than 50 percent of the mice. The highest dilution of the Standard shall protect less than 50 percent of the mice.

(6) The relative potency (RP) of the Unknown is determined by comparing the 50 percent endpoint dilution (highest bacterin dilution protecting 50 percent of the mice) of the Unknown with that of the Standard by the following formula:

$$\text{RP} = \frac{\text{reciprocal of 50 percent endpoint dilution of Unknown}}{\text{reciprocal of 50 percent endpoint dilution of Standard}}$$

(7) If the RP of the Unknown is less than 0.30, the serial being tested is unsatisfactory.

(8) If the 50 percent endpoint of an Unknown cannot be calculated because the lowest dilution does not exceed 50 percent protection, that serial may be retested in a manner identical to the initial test; *Provided*, That, if the Unknown is not retested or if the protection provided by the lowest dilution of the Standard exceeds the protection provided by the lowest dilution of the Unknown by six mice or more; or, if the total number of mice protected by the Standard exceeds the total number of mice protected by the Unknown by eight mice or more, the serial being tested is unsatisfactory.

(9) If the 50 percent endpoint of an Unknown in a valid test cannot be calculated because the highest dilution exceeds 50 percent protection, the Unknown is satisfactory without additional testing.

(10) If the RP is less than the minimum required in paragraph (c)(7) of this section, the serial may be retested by conducting two independent replicate tests in a manner identical to the initial test. The average of the RP